GPI-specific phospholipase D associates with an apoA-I- and apoA-IV-containing complex

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Abstract Glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD) is abundant in serum and associates with high density lipoproteins (HDL). We have characterized the distribution of GPI-PLD among lipoproteins in human plasma. Apolipoprotein (apo)-specific lipoproteins containing apoB (Lp[B]), apoA-I and A-II (Lp[A-I, A-II]), or apoA-I only (Lp[A-I]) were isolated using dextran sulfate and immunoaffinity chromatography. In six human plasma samples with HDL cholesterol ranging from 39 to 129 mg/dl, 79 ± 14% (mean ± SD) of the total plasma GPI-PLD activity was associated with Lp[A-I], 9 ± 12% with Lp[A-I, A-II], and 1 ± 1% with Lp[B]; and 11 ± 10% was present in plasma devoid of these lipoproteins. Further characterization of the GPI-PLD-containing lipoproteins by gel-filtration chromatography and nondenaturing polyacrylamide and agarose gel electrophoresis revealed that these apoA-I-containing particles/complexes were small (8 nm) and migrated with pre-β particles on agarose electrophoresis. Immunoprecipitation of GPI-PLD with a monoclonal antibody to GPI-PLD co-precipitated apoA-I and apoA-IV but little or no apoA-II, apoC-II, apoC-III, apoD, or apoE. In vitro, apoA-I but not apoA-IV or bovine serum albumin interacted directly with GPI-PLD, but did not stimulate GPI-PLD-mediated cleavage of a cell surface GPI-anchored protein. Thus, the majority of plasma GPI-PLD appears to be specifically associated with a small, discrete, and minor fraction of lipoproteins containing apoA-I and apoA-IV. —Deeg, M. A., E. L. Bierman, and M. C. Cheung. **GPI-specific phospholipase D associates with an apoA-I- and apoA-IV-containing complex. *J. Lipid Res.* 2001.** 42: **442–451.**

Supplementary key words lipoproteins • glycosylphosphatidylinositol • lipids • cholesterol • phospholipase D

The importance of minor, high density lipoprotein (HDL)-associated proteins in lipid metabolism and atherosclerosis has been increasingly appreciated (1–3). Some of these minor, HDL-associated proteins form specific, discrete particles [e.g., paraoxonase with apolipoprotein A-I (apoA-I) and J] or are broadly distributed among different HDL (e.g., platelet-activating factor acetylhydrolase). Glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD) associates with HDL (4). GPI-PLD is a 120-kDa N-glycosylated protein that is relatively abundant in human serum (5–10 μg of protein per ml) (5–9). GPI-PLD has been cloned from a number of sources, and GPI-PLD activity and mRNA have been identified in a number of tissues including mast cells, pancreatic islets, and liver (10, 11). Liver appears to be the primary source of circulating GPI-PLD (12, 13). Although GPI-PLD specifically cleaves GPIs in vitro, GPI-PLD appears to cleave GPI-anchored proteins from the cell surface only if the membrane is perturbed with detergents, raising the possibility that GPI-PLD is catalytically inactive in serum or requires a change in the membrane environment of the substrate to allow cleavage (14–16). ApoA-I has been reported to stimulate GPI-PLD activity in vitro, using a purified substrate (17), but it is unclear whether apoA-I is sufficient to stimulate GPI-PLD-mediated cleavage of a cell surface-bound GPI-anchored protein. Hence, the function of GPI-PLD in serum and why it associates with HDL are unknown.

As an initial approach to addressing the function of GPI-PLD in serum, we isolated apolipoprotein-specific lipoproteins and characterized GPI-PLD-containing lipoproteins in human plasma. Human plasma HDL are made up of two major populations of particles. Both contain apoA-I, but only one contains apoA-II (Lp[A-I] and Lp[A-I, A-II]) (18). The majority of GPI-PLD associates with Lp[A-I], with a size and electrophoretic mobility distinct from the bulk of HDL. This particle also contains apoA-IV. ApoA-I, but not apoA-IV, directly interacts with GPI-PLD but does not stimulate GPI-PLD-mediated cleavage of acetylcho-

Abbreviations: apo, apolipoprotein; BSA, bovine serum albumin; GPI, glycosylphosphatidylinositol; GPI-PLD, glycosylphosphatidylinositol-specific phospholipase D; HDL, high density lipoprotein; LDL, low density lipoprotein; Lp[A-I], lipoproteins containing apoA-I only; Lp[A-I, A-II], lipoproteins containing apoA-I and apoA-II; Lp[B], lipoproteins containing apoB; PAGE, polyacrylamide gel electrophoresis; PI-PLC, phosphatidylinositol phospholipase C; VLDL, very low density lipoprotein.

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linesterase (AChE, a GPI-anchored protein) from porcine erythrocytes.

EXPERIMENTAL PROCEDURES

Materials

Bovine serum albumin (BSA), fatty acid free, and polyethylene glycol (PEG, average molecular weight 8,000) were purchased from Sigma (St. Louis, MO). Dimethylsuberimidate (DMS) was purchased from Pierce (Rockford, IL). Anti-human apoC-II and apoC-III were purchased from Academy Bio-Medical (Houston, TX). Anti-human apoA-I and apoE were a kind gift from R. Lin (Indiana University, Indianapolis, IN). Purified human apoA-I and apoA-IV were a kind gift from J. Oram (University of Washington, Seattle, WA). Phosphatidylinositol phospholipase C (PI-PLC) was a kind gift from T. Rosenberry (Mayo Clinic, Jacksonville, FL). A monoclonal antibody to purified human serum GPI-PLD, 612c (19), was a gift from M. Davitz (Darby and Darby, New York, NY). Rabbit anti-GPI-PLD^{771} was prepared with a peptide corresponding to amino acids 771–790 of human serum GPI-PLD (M. A. Deeg, D. M. Larson, R. F. Bowen, M. A. Kennedy, and C. B. Verchere, unpublished observations). Human volunteers gave informed consent as approved by the respective institutional review boards of the University of Washington and Indiana University. Subjects were chosen among healthy individuals and patients with lipid disorders to obtain a broad range of conditions.

Preparation of lipoprotein particles

For apo-specific particles, blood from six fasting volunteers with HDL cholesterol ranging from 39 to 129 mg/dl was collected in tubes containing lithium heparin and passed sequentially through dextran sulfate cellulose, anti-apoA-II, and anti-apoA-I columns (20). The flowthrough plasma is free of apoA-I-, apoA-II-, and apoB-containing lipoproteins and is referred to as lipoprotein-free plasma. Elution of particles from the columns with 3 M sodium thiocyanate (NaSCN), pH 7.0, yields lipoproteins containing apoB (Lp[B]), apoA-II and A-I (Lp[A-I, A-II]), and apoA-I (Lp[A-I]), respectively. The eluted lipoproteins were immediately desalted on Sepharose G-25, and an aliquot was taken for determining GPI-PLD activity and concentrated. Recovery of GPI-PLD activity ranged from 80% to 110% (n = 6). Concentrating the sample resulted in a 10–15% loss of the GPI-PLD activity. Very low density lipoproteins (VLDL), low density lipoproteins (LDL), and HDL were prepared by ultracentrifugation (18). **Table 1** summarizes the total serum lipids and isolated apo-specific lipoprotein lipid composition for each of the subjects.

Purification and measurement of GPI-PLD activity

GPI-PLD was purified from human serum and activity was determined with [^{3}H]myristate-labeled variant surface glycoprotein as a substrate as previously described (21). Phosphatidic acid was confirmed as the only product by thin-layer chromatography (data not shown). Product formation was linear with respect to time and protein content. One unit of activity was arbitrarily defined as conversion of 1% of the substrate per minute.

Gel filtration

For gel filtration, aliquots of plasma (0.1 ml) or lipoproteins (0.4 ml) were loaded onto a Superose 12 HR 10/30 column or Superose 6 HR 10/30 (as indicated in figure legends; Amersham Pharmacia Biotech, Piscataway, NJ) in 10 mM Tris-HCl (pH 7.0), 100 mM NaCl, 0.02% NaN_3 with a flow rate of 0.25 ml/min; fractions (0.5 ml) were collected and aliquots were assayed for GPI-PLD activity or immunoreactive mass as described below. To determine whether the GPI-PLD complexes were detergent sensitive, Triton X-100 was added to human serum to a final concentration of 1% (v/v) and incubated overnight at 4°C. Lipoproteins were separated by gel filtration and GPI-PLD activity was determined in each fraction. Cholesterol content was determined with a cholesterol assay kit (Boehringer Mannheim, Indianapolis, IN) and apoA-I content was determined by radial immunodiffusion (22).

TABLE 1. Summary of total serum lipid values and lipoprotein composition for subjects

	Subject					
	1	2	3	4	5	6
Age	33	53	39	34	29	32
Gender	M	M[a]	F	M[b]	F[b]	M
Total cholesterol (mg/dl)	103	250	188	213	165	197
Total triglycerides (mg/dl)	41	50	44	179	70	233
VLDL cholesterol (mg/dl)	8	17	9	36	14	ND
LDL cholesterol (mg/dl)	54	104	108	126	86	ND
HDL cholesterol (mg/dl)	40	129	71	51	65	39
ApoA-I (mg/dl)	115	254	162	150	155	132
ApoB (mg/dl)	40	78	73	100	73	112
Lp[A-I]						
Cholesterol (mg/mg protein)	0.13	0.46	0.26	ND	ND	ND
Triglyceride (mg/mg protein)	0.09	0.03	0.02	ND	ND	ND
Lp [A-I, A-II]						
Cholesterol (mg/mg protein)	0.12	0.34	0.38	0.29	0.26	ND
Triglyceride (mg/mg protein	0.06	0.02	0.02	0.11	0.05	ND
Lp[B]						
Cholesterol (mg/mg protein)	0.43	0.62	0.43	1.06	ND	ND
Triglyceride (mg/mg protein)	0.41	0.27	0.13	0.75	ND	ND

Serum was isolated from six fasting subjects and serum lipids and apolipoproteins were determined as described in Materials and Methods. Apolipoprotein-specific lipoproteins were isolated, and cholesterol, triglycerides, and protein were measured as described in Experimetal Procedures. ND = not determined.

[a] Heterozygous familial hypercholesterolemia.

[b] Type 1 diabetes mellitus.

Electrophoresis and immunoblotting

Agarose electrophoresis and nondenaturing gradient polyacrylamide gel electrophoresis (PAGE) were performed as described previously (18). For sodium dodecyl sulfate (SDS)-PAGE, an aliquot was solubilized in Laemmli buffer (23) containing 1 mM dithiothreitol and electrophoresed in a 7.5% polyacrylamide gel. Purified apoA-I was used as a marker for pre-β particles and was identified by Western blotting.

For immunoblotting, SDS-PAGE, agarose, or nondenaturing gels were transferred to nitrocellulose. After blocking in phosphate-buffered saline containing 1% (w/v) BSA, 1% (w/v) milk protein, and 0.05% (v/v) Tween 20 for 1 h at room temperature, the membrane was incubated with 612c, a monoclonal antibody to the human serum form of GPI-PLD (19), at 15 μg/ml in the same buffer overnight at 4°C. The nitrocellulose was washed three times in blocking buffer and the antigen:antibody complex detected with ^{125}I-labeled goat anti-mouse (Amersham, Arlington Heights, IL). Either prestained (GIBCO-BRL, Gaithersburg, MD) or ^{125}I-labeled standards were used as molecular mass markers. Later experiments used rabbit anti-GPI-PLD^{771} to identify GPI-PLD (antibody used is listed in the figure legends). This antigen:antibody complex was identified with ECL Plus (Amersham Pharmacia Biotech).

Binding assay

To examine the interaction of GPI-PLD with proteins, we developed a gel-shift assay. Purified GPI-PLD (0.2 μg) was incubated with the desired protein (ranging from a 1- to 100-fold molar excess) in a final buffer consisting of 150 mM NaCl, 1 mM $CaCl_2$, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES, pH 7.0), and 0.1 mM phenylmethylsulfonyl fluoride in a final volume of 20 μl. After incubating for 60 min at 37°C, 12.5 μl of a 50% sucrose solution in electrophoresis buffer was added to the samples. The samples were loaded onto a 6% nondenaturing polyacrylamide gel. The gels were prerun for 20 min at 15 mA, the samples were loaded, and proteins were separated by running the gel for an additional 20 h at a constant 15 mA. Proteins were transferred to nitrocellulose and GPI-PLD was identified by Western blotting.

Purified GPI-PLD will aggregate (9). Incubating purified GPI-PLD with 2% Nonidet P-40 (NP-40) dissociates the aggregate and is referred to as monomeric GPI-PLD. The migration of the dissociated GPI-PLD relative to the aggregated GPI-PLD was measured with a Bio-Rad (Hercules, CA) Gel Doc system and the migration distance was determined with Molecular Analyst software (Bio-Rad). Serum GPI-PLD (0.5 μl of human serum) or GPI-PLD incubated with 2% NP-40 was used for comparison with the various experimental conditions. The migration of GPI-PLD under the various conditions relative to the aggregated GPI-PLD was compared with monomeric GPI-PLD and serum GPI-PLD, using the following equation: (migration distance of GPI-PLD − migration distance of serum GPI-PLD)/(migration of monomeric GPI-PLD − migration distance of serum GPI-PLD). Using this formula, the migration distance of GPI-PLD in the presence of NP-40 is 1 and the migration distance of serum GPI-PLD is 0.

To determine whether GPI-PLD was directly interacting with proteins, GPI-PLD (0.2 μg) was incubated with a 100-fold molar excess of protein as described above, and then proteins were cross-linked with DMS as previously described (24). GPI-PLD was identified by Western blotting, using anti-GPI-PLD^{771}.

Immunoprecipitation of GPI-PLD complexes

To immunoprecipitate GPI-PLD-containing complexes, 80 μl of 36% (w/v) PEG was added to 400 μl of human serum and incubated for 30 min on ice to remove VLDL and LDL. The sample was centrifuged (13,000 *g* for 20 min) and the supernatant was removed. To 25-μl aliquots of the supernatant, 5 μg of 612c or nonspecific mouse IgG_1 was added and incubated for 4 h at room temperature. Complexes were precipitated by adding 5 μg of rabbit anti-mouse IgG, incubated for 1 h at 4°C, and pelleted by centrifugation (13,000 *g* for 5 min). The precipitate was gently washed once with HEPES-buffered saline [20 mM HEPES (pH 7.0), 150 mM NaCl]. Apolipoprotein composition of the immunoprecipitate was determined by Western blotting. Either purified apolipoproteins or human serum was used as positive controls for the various apolipoproteins

Release of AChE from porcine erythrocytes

Release of GPI-anchored AChE from porcine erythrocytes was done as previously described (25). Isolated erythrocytes were incubated with GPI-PLD (0.5 μg) in the absence or presence of a 100-fold molar excess of apoA-I or A-IV. All preparations of GPI-PLD used in these experiments were tested for enzymatic activity, apoA-I binding, pattern of trypsin cleavage (21), and phosphorylation (M. A. Deeg, R. F. Bowen, M. A. Kennedy, and C. B. Verchere, unpublished observations) to verify proper biochemical properties of each preparation. Incubation with PI-PLC (1 U/ml) for 60 min at 37°C was used as a positive control. Release of AChE activity into the medium was determined by a modification of the method of Ellman et al. as previously described (25). Results are expressed as the rate of increase in absorbance at 412 nm.

Other analytical procedures

Protein was determined by the assay of Lowry et al. (26), with BSA as the standard. Triglycerides were determined with commercial kits (Sigma-Aldrich, St. Louis, MO). Apolipoprotein B was determined by a previously published immunoassay (27).

RESULTS

Distribution of GPI-PLD among lipoproteins in human plasma

The distribution of GPI-PLD among lipoproteins in human plasma was assessed by gel-filtration chromatography (Superose 12). Nearly all the GPI-PLD activity eluted in fractions 19–25, which overlapped with HDL. HDL eluted in fractions 20–25 (**Fig. 1A**). A small shoulder of activity preceding the major peak was present in all subjects. In some patients, a small amount of GPI-PLD activity was present at the void volume (Fig. 1A). To confirm that GPI-PLD activity correlated with GPI-PLD mass, gel-filtration fractions were subject to immunoblotting with a monoclonal antibody to human serum GPI-PLD, 612c. Immunoreactivity was identified in the fractions with the highest GPI-PLD enzymatic activity (Fig. 1B). Because lipoproteins can be disrupted by detergents, the effect of 1% Triton X-100 on GPI-PLD elution was examined. Using a different gel-filtration column (Superose 6 compared with Fig. 1A), GPI-PLD in serum eluted with HDL cholesterol (not shown) in fractions 36–46 (Fig. 1C), with the peak elution in fraction 40. After adding Triton X-100, GPI-PLD eluted in fractions 35–43, with the peak activity in fraction 38. This suggests that the Triton X-100:GPI-PLD complex may be larger than the native GPI-PLD complex in serum. These results are consistent with GPI-PLD associating with HDL in plasma.

To determine which HDL subfraction is associated with GPI-PLD, lipoproteins were isolated by a three-step dextran

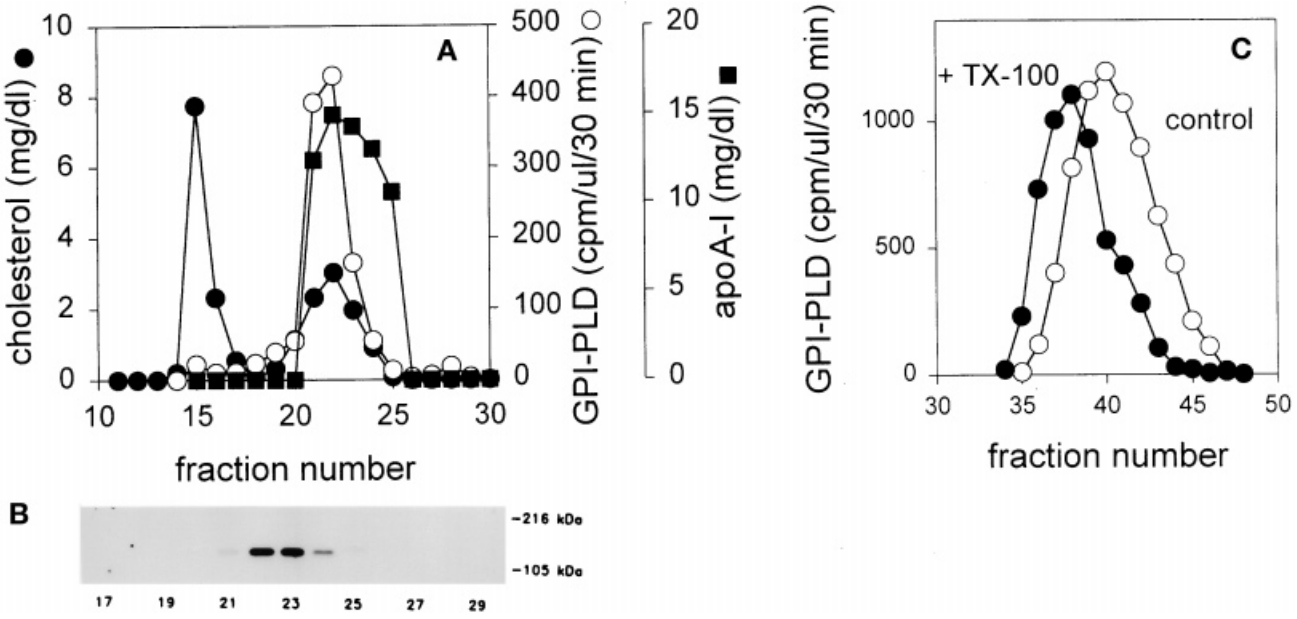

Fig. 1. Distribution of GPI-PLD among lipoproteins. A: Human plasma (patient 3, Table 1) was chromatographed by gel filtration (Superose 12). Aliquots were taken for the determination of GPI-PLD activity (open circles), apoA-I (squares), and cholesterol (closed circles) as described in Experimental Procedures. The absorbance at 280 nm was omitted for clarity. The first peak of cholesterol corresponds to VLDL and LDL and the second to HDL. B: GPI-PLD mass was determined by Western blotting, using 612c as the antibody as described in Experimental Procedures. Results are representative of plasma samples from six subjects. C: Human plasma (subject 1, Table 1) was incubated overnight in the absence (control) or presence of 1% Triton X-100 (+TX-100), and then lipoproteins were separated by gel filtration (Superose 6). Only the fractions corresponding to HDL are shown. Fractions were assayed for GPI-PLD activity as described above.

sulfate and immunoaffinity chromatography procedure, which isolates lipoproteins containing apoB (Lp[B]), apoA-I without apoA-II (Lp[A-I]), and apoA-I with apoA-II (Lp[A-I, A-II]). The remaining fraction is essentially free of lipoproteins. Fractionation was performed on samples from six individuals with HDL cholesterol ranging from 39 to 129 mg/dl (Table 1). The majority of GPI-PLD activity was isolated with Lp[A-I] (79 ± 14%). A small and variable amount was isolated with Lp[A-I, A-II] (9 ± 12%), Lp[B] (1 ± 1%), or lipoprotein-free plasma (11 ± 10%) (**Table 2**). These results are consistent with the gel-filtration results, with GPI-PLD coeluting with HDL, and suggest that the majority of GPI-PLD in serum is associated with Lp[A-I] particles. GPI-PLD in the lipoprotein-free fraction may be "free," that is, not bound to lipoproteins, not associated with other proteins, or partially proteolytically cleaved, or it may represent GPI-PLD that dissociates from lipoproteins during the isolation of the particles.

TABLE 2. Distribution of GPI-PLD among lipoproteins

Patient	Total Serum GPI-PLD	GPI-PLD Distribution (% of total)			
		Lp[A-I]	Lp[A-I, A-II]	Lp[B]	Lp-Free
	U/l				
1	10.5	82	4.4	0	13.5
2	6.5	69	2.6	0	28.4
3	9.6	91	4.7	0.4	3.9
4	8.7	74	7.9	3.6	14.2
5	6.2	59	33	1.2	6.9
6	7.4	98	1.5	0	0.4
Mean ± SD		79 ± 14	9 ± 12	1 ± 1	11 ± 10

Lipoproteins were isolated from six individuals as described in Experimental Procedures, and distribution of GPI-PLD activity was determined.

Characterization of GPI-PLD-containing lipoproteins

Lp[A-I] and Lp[A-I, A-II] were isolated and GPI-PLD-containing complexes were further characterized by gel-filtration chromatography, nondenaturing PAGE, and agarose gel electrophoresis. On gel filtration, Lp[A-I] had only one peak of GPI-PLD activity (**Fig. 2A**). In contrast, in subjects with GPI-PLD activity in Lp[A-I, A-II], there were two peaks of GPI-PLD activity (Fig. 2B). The larger peak had an elution volume similar to the GPI-PLD activity in Lp[A-I] whereas the minor peak of activity observed had a smaller elution volume. This minor peak corresponded to the shoulder of activity seen in the gel filtration of plasma (Fig. 1A). In the lipoprotein-free plasma, the GPI-PLD activity had a slightly greater elution volume than was observed in Lp[A-I] (Fig. 2A).

When the two populations of apoA-I-containing lipoproteins were separated by nondenaturing gradient PAGE, Coomassie blue staining showed that the component particles were heterogeneous in size (data not shown). However, in both Lp[A-I] and Lp[A-I, A-II], the only particles containing GPI-PLD were seen at 8 nm (Fig. 2B). This corresponded to a similarly sized GPI-PLD complex in plasma (Fig. 2B). This particular individual (subject 2; Table 1) also has a significant amount of GPI-PLD migrating at the dye front consistent with the large amount of GPI-PLD ac-

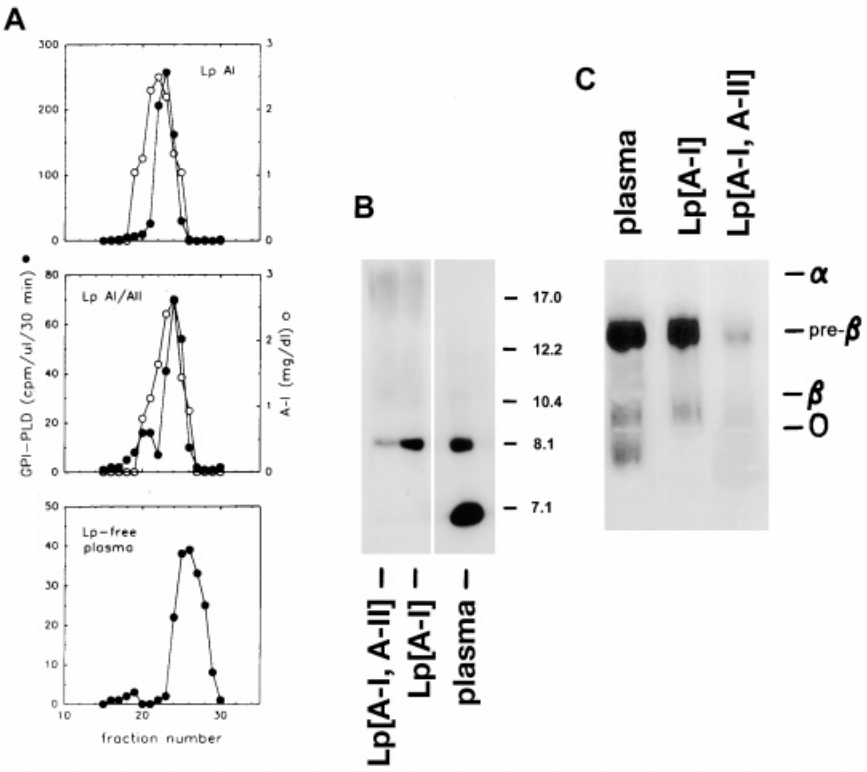

Fig. 2. Characterization of GPI-PLD-containing lipoproteins. Lipoprotein particles prepared by affinity chromatography. A: Immunoaffinity-purified lipoprotein particles and Lp-free plasma were analyzed by gel filtration (Superose 6) and fractions were analyzed for GPI-PLD activity (closed circles) and apoA-I (open circles) as in Fig. 1. No apoA-I or cholesterol was detected in the Lp-free plasma. Cholesterol and absorbance at 280 nm were omitted for clarity. Results are from subject 2, Table 1 and are representative of samples from two other subjects (1 and 4, Table 1). B: Aliquots (20 μg of apoA-I) of Lp[A-I] and Lp[A-I, A-II] or 5 μl of plasma were separated by nondenaturing PAGE and immunoblotted with 612c as described in Experimental Procedures. Standards include thyroglobulin (17.0 nm), ferritin (12.2 nm), catalase (10.4 nm), lactate dehydrogenase (8.1 nm), and BSA (7.1 nm). Results are representative of separate experiments from two subjects (subjects 1 and 2; Table 1). Weak staining around 17 nm is nonspecific (not shown). C: An aliquot (5 μl) of plasma or lipoprotein particles was separated by agarose electrophoresis and immunoblotted with anti-GPI-PLD[771] as described in Experimental Procedures. Migration of HDL (α) and LDL (β) particles was determined by lipid staining of a separate gel run in parallel. Purified apoA-I was used to identified pre-β particles. The origin (O) is indicated.

tivity present in the lipoprotein-free fraction in this subject (Table 2). These results suggest that GPI-PLD distribution between Lp[A-I] and Lp[A-I, A-II] is restricted to a relatively small particle or complex distinct from the bulk of HDL. This is supported by the observation that GPI-PLD-containing lipoproteins migrated with pre-β particles on agarose electrophoresis (Fig. 2C).

Apolipoprotein composition of GPI-PLD-containing complexes

To examine whether apolipoproteins other than A-I are associated with the GPI-PLD-containing complex, we immunoprecipitated GPI-PLD from two human plasma samples and probed for additional apolipoproteins, using Western blotting. With the control antibodies, small amounts of apoA-I and apoE were detected, suggesting some nonspecific precipitation with the nonspecific antibodies. As expected, 612c, a monoclonal antibody to human serum GPI-PLD, immunoprecipitated GPI-PLD and a significant amount of apoA-I from human plasma (**Fig. 3**). Interestingly, apoA-IV was also detected. However, no immunoreactivity for apoA-II, apoC-II, apoC-III, or apoD was detected. A similar amount of apoE was detected in precipitates using either nonspecific antibodies or 612c.

Interaction between GPI-PLD and apoA-I and apoA-IV

To determine whether GPI-PLD directly interacts with apoA-I or apoA-IV, we developed a gel-shift assay utilizing a nondenaturing gel to separate GPI-PLD complexes. GPI-PLD was identified by Western blotting. Purified GPI-PLD migrated as a single band (**Fig. 4A**, lane 2). This likely represents aggregated GPI-PLD that forms during purification (9). Aggregated GPI-PLD can be dissociated by adding detergent (4). In the presence of NP-40, a faster migrating band, which likely represents monomeric GPI-PLD, is present (Fig. 4A, lane 1). In contrast, a single GPI-PLD complex was identified in serum, which migrated slower than the monomeric GPI-PLD (Fig. 4A, lane

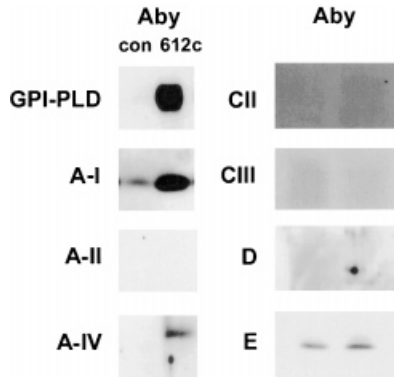

Fig. 3. Immunoprecipitation of GPI-PLD complexes from human plasma. Polyethylene glycol-treated human serum was incubated with nonspecific mouse IgG_1 (control, con) or 612c and complexes were precipitated as described in Experimental Procedures. Precipitates were probed for the indicated apolipoprotein by Western blotting. Anti-GPI-PLD[771] was used to identify GPI-PLD. Positive controls were purified protein or human serum (data not shown). Results are from subject 1 in Table 1, with similar results with plasma from subject 3 in Table 1. Aby, Antibody.

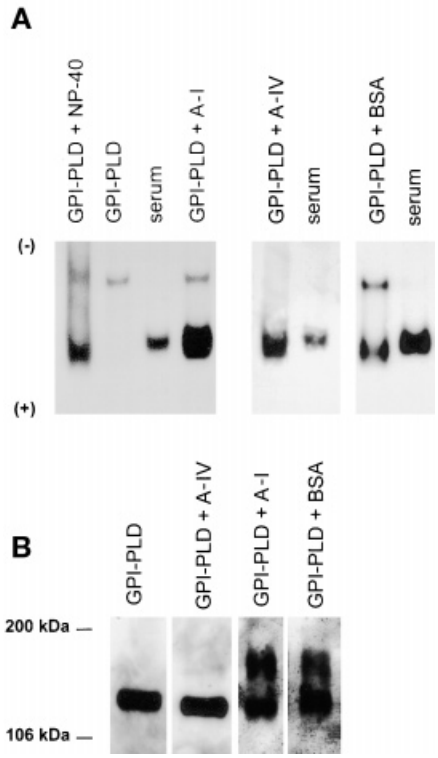

Fig. 4. GPI-PLD binding to apoA-I, apoA-IV, and BSA. A: Purified GPI-PLD (0.2 μg) was incubated in the absence or presence of NP-40 (+ NP-40), apoA-I (100-fold molar excess), apoA-IV (100-fold molar excess), or BSA (100-fold molar excess) and complexes were separated by nondenaturing gel electrophoresis. GPI-PLD was identified by Western blotting as described in Experimental Procedures. Human serum (0.5 μl) was used for comparison. The cathode (+) and anode (−) are indicated. B: GPI-PLD was incubated in the absence or presence of apoA-I (100-fold molar excess), apoA-IV (100-fold molar excess), or BSA (100-fold molar excess) and proteins were cross-linked with DMS. Proteins were separated by SDS-PAGE and GPI-PLD was identified by Western blotting with anti-GPI-PLD[771]. DMS did not alter the immunoreactivity of GPI-PLD (data not shown). Molecular mass standards are indicated.

3). ApoA-I and BSA also disaggregate purified GPI-PLD (17). In the presence of a 100-fold molar excess of apoA-I, both forms of GPI-PLD were present (Fig. 4A, lane 4): the aggregated GPI-PLD and a faster-migrating GPI-PLD band with mobility comparable to the GPI-PLD complex in serum. This suggests that apoA-I interacts directly with GPI-PLD. Although the same amount of GPI-PLD was used in the absence or presence of apoA-I, there was a large increase in the total immunoreactivity in the presence of apoA-I (compare lanes 2 and 4, Fig. 4A). One possible explanation is a nonspecific effect of adding excess protein, which may enhance recovery or transfer efficiency of monomeric GPI-PLD. However, this does not appear to be the case because *i*) adding nonspecific IgG to the binding mixture either in the absence or presence of apoA-I does affect the results and *ii*) if ^{125}I-labeled GPI-PLD is used in the binding assay, similar results are obtained (data not shown). This discrepancy is likely due to the inefficient transfer of aggregated GPI-PLD out of the gel and onto the nitrocellulose (data not shown). Finally, dissociation may also induce a conformational change in GPI-PLD resulting in increased immunoreactivity.

To confirm that apoA-I directly interacts with GPI-PLD, GPI-PLD and apoA-I (100-fold molar excess) were incubated together, and then DMS was added to cross-link interacting proteins. In the absence of apoA-I, the native GPI-PLD has an apparent molecular mass of 120 kDa on SDS-PAGE (Fig. 4B, lane 1). In the presence of apoA-I (28 kDa), a new band with an apparent molecular mass of 141 kDa was identified with the GPI-PLD antibody after cross-linking. These results are consistent with a direct interaction between apoA-I and GPI-PLD with a 1:1 stoichiometry.

To determine the specificity of apoA-I interaction with GPI-PLD, the interaction between GPI-PLD and apoA-IV and BSA was examined. Both apoA-IV and BSA were able to dissociate GPI-PLD as determined by the gel-shift assay (Fig. 4A). However, the mobility of the dissociated GPI-PLD was faster than the serum GPI-PLD complex and similar to monomeric GPI-PLD. This raises the possibility that apoA-IV and BSA are either forming a different complex with GPI-PLD than forms with apoA-I, or that these proteins may simply act as "detergents" to dissociate aggregated GPI-PLD without forming a direct interaction with GPI-PLD. This is supported by the observation that in contrast to apoA-I, apoA-IV, in 100-fold excess, does not cross-link with GPI-PLD (Fig. 4B). However, BSA did cross-link with GPI-PLD (Fig. 4B), but the cross-linked product was identical in size to the apoA-I-GPI-PLD cross-linked product. This raises the possibility that the cross-linked product may represent apoA-I, which is commonly present in commercial preparations of BSA.

To further characterize the GPI-PLD interaction with apoA-I, we examined the concentration dependence of apoA-I interaction with GPI-PLD. With increasing amounts of apoA-I, there was an increase in the amount of monomeric GPI-PLD, consistent with dissociation of aggregated

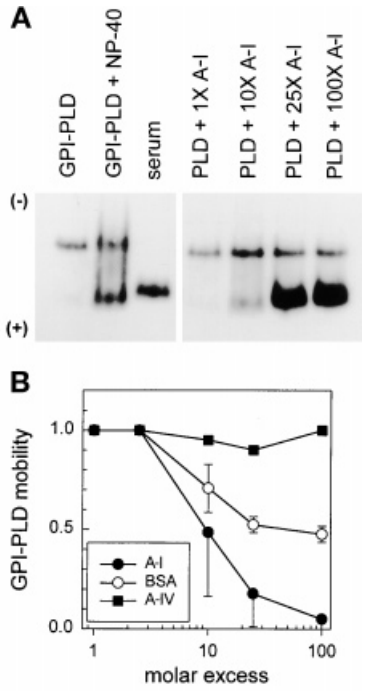

Fig. 5. Concentration dependence of apoA-I binding to GPI-PLD. GPI-PLD (0.2 μg) was incubated with increasing amounts of apoA-I (A), A-IV, or BSA (1- to 100-fold molar excess) and GPI-PLD complexes were separated by nondenaturing gel electrophoresis and identified by Western blotting as described in the legend to Fig. 4. The cathode (+) and anode (−) are indicated. B: Results from three independent experiments ± SD. Mobility of monomeric GPI-PLD and GPI-PLD complexes in serum correspond to mobilities of 1 and 0, respectively. The formula for calculating mobilities is discussed in Experimental Procedures.

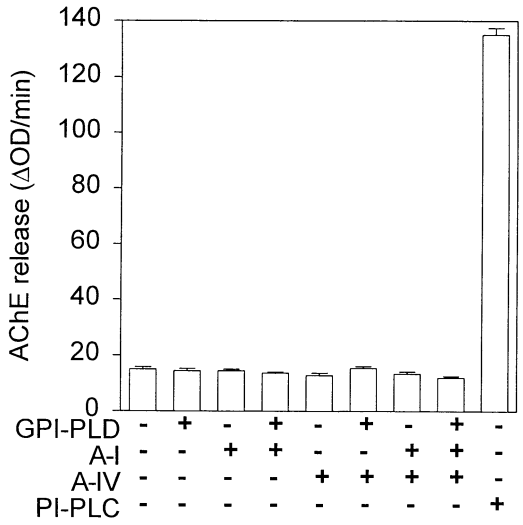

Fig. 6. Effect of GPI-PLD on release of acetylcholinesterase (AChE) from porcine erythrocytes. Porcine erythrocytes were isolated and incubated with buffer; GPI-PLD (0.5 μg); GPI-PLD (0.5 μg) in the presence of a 100-fold molar excess of apoA-I, A-IV, or both together; or phosphatidylinositol phospholipase C (PI-PLC) (1 U/ml). Medium content of AChE was determined after a 60-min incubation as described in Experimental Procedures. Results are means ± SD from three independent experiments.

GPI-PLD (**Fig. 5A**). At low molar ratios, GPI-PLD migrated with the same mobility as monomeric GPI-PLD. With increasing concentrations of apoA-I, there was a decrease in the mobility of GPI-PLD. At a 100-molar excess, the apoA-I:GPI-PLD complex had a mobility similar to that of the GPI-PLD complex in serum (Fig. 5A). These results are summarized in Fig. 5B (a mobility identical to monomeric GPI-PLD is equivalent to a value of 1, whereas a mobility identical to the GPI-PLD complex in serum has a value of 0). The 50% effective concentration was equivalent to a 10-fold excess of apoA-I, similar to previous reports utilizing a different assay to measure the apoA-I:GPI-PLD interaction (17).

Consistent with the cross-linking data, apoA-IV did not alter the mobility of GPI-PLD (Fig. 5B). In contrast, higher molar concentrations (≥10-fold excess) of BSA did alter the mobility of GPI-PLD, but not as effectively as apoA-I (Fig. 5B).

Effect of apoA-I on GPI-PLD-mediated release of GPI-anchored proteins from intact cells

Purified GPI-PLD does not release GPI-anchored proteins from the cell surface (14–16). To determine whether apoA-I and/or apoA-IV enhances GPI-PLD-mediated release of a GPI-anchored protein from an intact cell, the release of AChE from porcine erythrocytes was examined. A GPI-PLD complex with apoA-I and/or apoA-IV was formed as described above and then added to porcine erythrocytes. Under basal conditions, a small amount of AChE is released into the medium (**Fig. 6**). Adding PI-PLC causes a significant release of AChE. However, GPI-PLD in the absence or presence of apoA-I did not cause a significant release of AChE. These results suggest that apoA-I and apoA-IV are not sufficient for GPI-PLD cleavage of a cell surface GPI-anchored protein.

DISCUSSION

HDL is a heterogeneous population of lipoprotein particles differing in protein and lipid composition. Minor HDL-associated proteins play important roles in lipid metabolism and/or atherosclerosis. Some of these proteins are associated with a variety of HDL particles whereas others form a specific HDL of a particular lipid and protein composition. We report here that the majority of GPI-PLD in humans associates with a specific HDL lipoprotein containing apoA-I and apoA-IV.

Apolipoproteins associated with lipoproteins generally have hydrophobic domains that allow interaction with lipids. GPI-PLD is amphiphilic (4) and it is intuitive that GPI-PLD may associate with lipids simply on the basis of hydrophobic interaction. Some HDL-associated proteins, for example, lecithin:cholesterol acyltransferase, cholesterol ester transfer protein, and paraoxonase, are found predominately on Lp[A-I] particles, whereas apoE, apoD, and apoC are found on Lp[A-I, A-II] (28–30). GPI-PLD

forms primarily a small, discrete particle with the predominant GPI-PLD complex containing apoA-I and apoA-IV. This complex represents a minor component of the total apoA-I in plasma. The concentration of GPI-PLD in human plasma has been estimated at 5–10 μg/ml (31). Given that the apoA-I concentration is approximately 1 mg/ml and assuming a 1:1 stoichiometry of interaction, GPI-PLD is associated with no more than 0.2% of the total apoA-I. From the data presented here, it cannot be determined whether this represents a protein complex or truly a lipoprotein particle.

The limited distribution of GPI-PLD to Lp[A-I] also suggests that there is a unique feature of this particle/complex that accounts for the restricted distribution of GPI-PLD. Our binding studies suggest that protein interaction with purified GPI-PLD may involve two different steps. The first step is to dissociate the aggregated GPI-PLD. This may likely represent a nonspecific hydrophobic interaction because apoA-I, apoA-IV, and BSA, but not IgG (data not shown), dissociate the aggregated GPI-PLD into monomers. This is consistent with previously published work showing that both apoA-I and BSA can dissociate aggregated GPI-PLD (17). The second step is a direct interaction between the protein and GPI-PLD that can be identified by cross-linking and a change in the mobility of the GPI-PLD complex. This appears to be specific for apoA-I because we were unable to demonstrate apoA-IV cross-linking to GPI-PLD or changes in GPI-PLD mobility on a nondenaturing gel. This is the first demonstration of a difference between apolipoproteins in interacting with GPI-PLD. Incubating GPI-PLD with BSA also altered the mobility of GPI-PLD on a nondenaturing gel and identified a cross-linked protein. However, the cross-linked protein was identical in mass (141 kDa) to the product formed between apoA-I and GPI-PLD. Hence it is possible that the apoA-I contamination present in most commercial BSA preparations may account for some or all of the effects seen with BSA in these experiments.

These results raise the possibility of a specific apoA-I-binding site on GPI-PLD. Consistent with this is our observation that the phosphorylation of GPI-PLD inhibits binding to apoA-I but not GPI-PLD-mediated inhibition of islet amyloid polypeptide fibril formation (M. A. Deeg, D. M. Larson, R. F. Bowen, M. A. Kennedy, and C. B. Verchere, unpublished observations). Interestingly, this differs from the interaction between paraoxonase and apoA-I, where it appears that apoA-I induces a specific lipid-binding domain. Paraoxonase appears to bind to the induced lipid domain and not directly with apoA-I (32). Although apoA-IV also immunoprecipitated with 612c, apoA-IV did not interact directly with GPI-PLD. This suggests that the apoA-IV associated with the GPI-PLD-containing particle may interact with the lipids or some other component of the particle. The specific interaction of apoA-I with GPI-PLD could result from the conformation of apoA-I. ApoA-I conformation is influenced by the lipid composition of the particle (33, 34) or other proteins that could alter the interaction of apoA-I with GPI-PLD. This may explain the relatively smaller amount of GPI-PLD associated with Lp[A-I, A-II].

There appear to be two populations of Lp[A-I, A-II] that contain GPI-PLD. The major population has characteristics similar to those of the GPI-PLD associated with Lp[A-I], suggesting a similar composition. The minor population of GPI-PLD in Lp[A-I, A-II] is larger by gel filtration (Fig. 3) and may contain more than one molecule of apoA-I, apoA-II, or GPI-PLD per particle. Alternatively, this minor fraction may contain more lipids or other proteins such as apoE. We did not detect apoA-II in the immunoprecipitates with 612c. It is likely that the apoA-II present in the immunoprecipitate may have been below the level of detection because we used serum from individuals with low amounts of GPI-PLD in the Lp[A-I, A-II] fraction. The composition of the GPI-PLD-containing particles will require a large-scale isolation of GPI-PLD-containing particles.

The role of apoA-I in the function of GPI-PLD is still unclear. Adding apoA-I or apoA-IV to GPI-PLD did not stimulate cleavage of AChE from porcine erythrocytes. This is consistent with earlier observations that purified GPI-PLD or whole serum does not cleave GPI-anchored proteins from cells unless detergent is added to the cells or isolated membranes (14–16). The resistance of GPI-anchored proteins to cleavage by GPI-PLD results from the membrane environment of the GPI-anchored protein (14). Although we originally hypothesized that apoA-I might perturb the plasma membrane, apoA-I and/or apoA-IV is insufficient to stimulate GPI-PLD cleavage of GPI-anchored proteins. If serum GPI-PLD does cleave cell surface GPI-anchored proteins, then it must be regulated, because enough GPI-PLD is present in serum to cleave all the surface GPI-anchored proteins in minutes (11). Another speculation is that apoA-I directs delivery of GPI-PLD and the lipoprotein-associated GPI-anchored proteins (35, 36) to a specific locale, either extracellular or intracellular, for release of the protein and/or phosphatidic acid, both of which may be biologically active. Obviously, if circulating GPI-PLD is involved in cleaving GPI-anchored proteins, the mechanism is much more complex than simply presenting the protein to the cell. Finally, it is possible, too, that the function of GPI-PLD in serum may involve other interprotein interactions independent of its catalytic activity similar to lipoprotein lipase and hepatic lipase (37, 38).

The lack of an effect of apoA-I on GPI-PLD activity appears to be at odds with previous published work demonstrating that apoA-I stimulated GPI-PLD activity in vitro (17). Those studies utilized a purified substrate in a detergent (Triton X-100) micelle rather than release of a GPI-anchored protein from intact cells. ApoA-I stimulated GPI-PLD under those conditions only when the detergent concentration in the assay was near the critical micelle concentration. This effect was not specific to apoA-I because apoA-II and apoA-IV and, to a lesser extent, BSA also stimulated GPI-PLD activity under the same conditions (17). We, too, have noted that apoA-I affected GPI-PLD activity near the critical micelle concentration of NP-40, but the effect was variable: in some experiments apoA-I stimulated and in others inhibited GPI-PLD activity as much as ±50% (data not shown). Together, these results

suggest that the apolipoprotein effects on GPI-PLD activity, when measured near the critical micelle concentration of the detergent in the assay, may derive from nonspecific hydrophobic interactions and/or lipid contaminants in the protein preparations. Although each preparation of GPI-PLD used in these experiments is examined for expected biochemical properties (enzymatic activity, phosphorylation, and trypsin cleavage pattern as well as apoA-I binding), we cannot eliminate the possibility that the conformation of GPI-PLD is subtly altered with purification as occurs with apoE (39). Regardless, the debate about whether GPI-PLD is enzymatically active in serum is tempered by the observation that bicarbonate, at concentrations present in serum, inhibits GPI-PLD activity via carbamylation (40).

In summary, GPI-PLD forms a small, discrete complex with apoA-I and apoA-IV in human serum. ApoA-I directly interacts with GPI-PLD but does not stimulate GPI-PLD-mediated release of a GPI-anchored protein from erythrocytes. Although the function of GPI-PLD is unknown, preliminary data demonstrate that GPI-PLD also exchanges between HDL and VLDL in the postprandial state and that GPI-PLD enhances VLDL-binding to hepatocytes (M. A. Deeg and R. F. Bowen, unpublished observations). Many HDL-associated proteins involved in VLDL metabolism, including apoC, apoE, and apoA-IV, exchange between HDL and VLDL in the postprandial state. Further experiments are required to examine this potential role of GPI-PLD in lipid metabolism.

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REFERENCES

1. Jordan-Starck, T. C., S. D. Lund, D. P. Witte, B. J. Aronow, C. A. Ley, W. D. Stuart, D. K. Swertfeger, L. R. Clayton, S. F. Sells, B. Paigen, and J. A. K. Harmony. 1994. Mouse apolipoprotein J: characterization of a gene implicated in atherosclerosis. *J Lipid Res.* **35:** 194–210.
2. Shih, D. M., L. Gu, Y. R. Xia, M. Navab, W. F. Li, S. Hama, L. W. Castellani, C. E. Furlong, L. G. Costa, A. M. Fogelman, and A. J. Lusis. 1998. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature.* **394:** 284–287.
3. Navab, M., S. Y. Hama, G. P. Hough, C. C. Hedrick, R. Sorenson, B. N. La Du, J. A. Kobashigawa, G. C. Fonarow, J. A. Berliner, H. Laks, and A. M. Fogelman. 1998. High density associated enzymes: their role in vascular biology. *Curr. Opin. Lipidol.* **9:** 449–456.
4. Hoener, M. C., and U. Brodbeck. 1992. Phosphatidylinositol-glycan-specific phospholipase D is an amphiphilic glycoprotein that in serum is associated with high-density lipoproteins. *Eur. J. Biochem.* **206:** 747–757.
5. Scallon, B. J., W-J. C. Fung, T. C. Tsang, S. Li, H. Kado-Fong, K-S. Huang, and J. P. Kochan. 1991. Primary structure and functional activity of a phosphatidylinositol-glycan-specific phospholipase D. *Science.* **252:** 446–448.
6. Tsang, T. C., W. J. Fung, J. Levine, C. N. Metz, M. A. Davitz, and D. K. Burns. 1992. Isolation and expression of two human glycosylphosphatidylinositol phospholipase D (GPI-PLD) cDNAs. *FASEB J.* **6:** A1922.
7. LeBoeuf, R. C., M. Caldwell, Y. Guo, C. Metz, M. A. Davitz, L. K. Olson, and M. A. Deeg. 1998. Mouse glycosylphosphatidylinositol-specific phospholipase D (Gpld1) characterization. *Mamm. Genome.* **9:** 710–714.
8. Davitz, M. A., D. Hereld, S. Shak, J. Krakow, P. T. Englund, and V. Nussenzweig. 1987. A glycan-phosphatidylinositol-specific phospholipase D in human serum. *Science.* **238:** 81–84.
9. Huang, K-S., S. Li, W-J. C. Fung, J. D. Hulmes, L. Reik, Y-C. E. Pan, and M. G. Low. 1990. Purification and characterization of glycosylphosphatidylinositol-specific phospholipase D. *J. Biol. Chem.* **265:** 17738–17745.
10. Deeg, M. A., and M. A. Davitz. 1994. Structure and function of the glycosylphosphatidylinositol-specific phospholipase D. *In* Signal-Activated Phospholipases. M. Liscovitch, editor. R. G. Landes Company, Austin, TX. 125–138.
11. Low, M. G. Structure and function of GPI-specific phospholipases. 2000. *In* PNH and the GPI-Linked Proteins. Academic Press, San Diego, CA. 239–268.
12. Maguire, G. A., and A. Gossner. 1995. Glycosyl phosphatidyl inositol phospholipase D activity in human serum. *Annu. Clin. Biochem.* **32:** 74–78.
13. Rhode, H., E. Lopatta, M. Schulze, C. Pascual, H. P. Schulze, K. Schubert, H. Schubert, K. Reinhart, and A. Horn. 1999. Glycosylphosphatidylinositol-specific phospholipase D in blood serum: is the liver the only source of the enzyme? *Clin. Chim. Acta.* **281:** 127–145.
14. Low, M. G., and K-S. Huang. 1991. Factors affecting the ability of glycosylphosphatidylinositol-specific phospholipase D to degrade the membrane anchors of cell surface proteins. *Biochem. J.* **279:** 483–493.
15. Bergman, A-S., and S. R. Carlsson. 1994. Saponin-induced release of cell-surface-anchored Thy-1 by serum glycosylphosphatidylinositol-specific phospholipase D. *Biochem. J.* **298:** 661–668.
16. Deng, J. T., M. F. Hoylaerts, M. E. De Broe, and V. O. van Hoof. 1996. Hydrolysis of membrane-bound liver alkaline phosphatase by GPI-PLD requires bile salts. *Am. J. Physiol.* **271:** G655–G663.
17. Hoener, M. C., R. Golli, and U. Brodbeck. 1993. Glycosyl-phosphatidylinositol-specific phospholipase D: interaction with and stimulation by apolipoprotein A-I. *FEBS Lett.* **327:** 203–206.
18. Cheung, M. C., and J. J. Albers. 1984. Characterization of lipoprotein particles isolated by immunoaffinity chromatography. Particles containing A-I and A-II and particles containing A-I but no A-II. *J. Biol. Chem.* **259:** 12201–12209.
19. Metz, C. N., P. Thomas, and M. A. Davitz. 1992. Immunolocalization of a glycosylphosphatidylinositol-specific phospholipase D in mast cells found in normal tissue and neurofibromatosis lesions. *Am. J. Pathol.* **140:** 1275–1281.
20. Cheung, M. C., and A. C. Wolf. 1989. In vitro transformation of apoA-I-containing lipoprotein subpopulations: role of lecithin: cholesterol acyltransferase and apoB-containing lipoproteins. *J. Lipid Res.* **30:** 499–509.
21. Deeg, M. A., and R. F. Bowen. 1999. Midportion antibodies stimulate glycosylphosphatidylinositol-specific phospholipase D activity. *Arch. Biochem. Biophys.* **370:** 278–284.
22. Cheung, M. C., and J. J. Albers. 1977. The measurement of apolipoprotein A-I and A-II levels in men and women by immunoassay. *J. Clin. Invest.* **60:** 43–50.
23. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
24. Swaney, J. B. 1986. Use of Cross-Linking Reagents to Study Lipoprotein Structure. Academic Press, Orlando, FL.
25. Low, M. G., and J. B. Finean. 1977. Non-lytic release of acetylcholinesterase from erythrocytes by a phosphatidylinositol-specific phospholipase C. *FEBS Lett.* **82:** 143–146.
26. Lowry, O. H., A. L. Rosebrough, L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
27. Albers, J. J., V. G. Cabana, and W. R. Hazzard. 1975. Immunoassay of human plasma apolipoprotein B. *Metabolism.* **24:** 1339–1351.
28. Cheung, M. C., and J. J. Albers. 1982. Distribution of high density lipoprotein particles with different apoprotein composition: particles with A-I and A-II and particles with A-I but no A-II. *J. Lipid Res.* **23:** 747–753.

29. James, R. W., D. Hochstrasser, J. D. Tissot, M. Funk, R. Appel, F. Barja, C. Pellegrini, A. F. Muller, and D. Pometta. 1988. Protein heterogeneity of lipoprotein particles containing apolipoprotein A-I without apolipoprotein A-II and apolipoprotein A-I with apolipoprotein A-II isolated from human plasma. *J. Lipid Res.* **29:** 1557–1571.
30. Duverger, N., D. Rader, P. Duchateau, J. C. Fruchart, G. Castro, and H. B. Brewer, Jr. 1993. Biochemical characterization of the three major subclasses of lipoprotein A-I preparatively isolated from human plasma. *Biochemistry.* **32:** 12372–12379.
31. Davitz, M. A., J. Hom, and S. Schenkman. 1989. Purification of a glycosyl-phosphatidylinositol-specific phospholipase D from human plasma. *J. Biol. Chem.* **264:** 13760–13764.
32. Sorenson, R. C., C. L. Bisgaier, M. Aviram, C. Hsu, S. Billecke, and B. N. La Du. 1999. Human serum paraoxonase/arylesterase's retained hydrophobic N-terminal leader sequence associates with HDLs by binding phospholipids: apolipoprotein A-I stabilizes activity. *Arterioscler. Thromb. Vasc. Biol.* **19:** 2214–2225.
33. Sparks, D. L., M. C. Phillips, and S. Lund-Katz. 1992. The conformation of apolipoprotein A-I in discoidal and spherical recombinant high density lipoprotein particles. 13C NMR studies of lysine ionization behavior. *J. Biol. Chem.* **267:** 25830–25838.
34. Sparks, D. L., W. S. Davidson, S. Lund-Katz, and M. C. Phillips. 1993. Effect of cholesterol on the charge and structure of apolipoprotein A-I in recombinant high density lipoprotein particles. *J. Biol. Chem.* **268:** 23250–23257.
35. Vakeva, A., M. Jauhiainen, C. Ehnholm, T. Lehto, and S. Meri. 1994. High-density lipoproteins can act as carriers of glycophosphoinositol lipid-anchored CD59 in human plasma. *Immunology.* **82:** 28–33.
36. Lesma, E., J. Moss, H. B. Brewer, Jr., R. Bortell, D. Greiner, J. Mordes, and A. A. Rossini. 1998. Characterization of high density lipoprotein-bound and soluble RT6 released following administration of anti-RT6.1 monoclonal antibody. *J. Immunol.* **161:** 1212–1219.
37. Merkel, M., Y. Kako, H. Radner, I. S. Cho, R. Ramasamy, J. D. Brunzell, I. J. Goldberg, and J. L. Breslow. 1998. Catalytically inactive lipoprotein lipase expression in muscle of transgenic mice increases very low density lipoprotein uptake: direct evidence that lipoprotein lipase bridging occurs in vivo. *Proc. Natl. Acad. Sci. USA.* **95:** 13841–13846.
38. Dichek, H. L., W. Brecht, J. Fan, Z. S. Ji, S. P. McCormick, H. Akeefe, L. Conzo, D. A. Sanan, K. H. Weisgraber, S. G. Young, J. M. Taylor, and R. W. Mahley. 1998. Overexpression of hepatic lipase in transgenic mice decreases apolipoprotein B-containing and high density lipoproteins. Evidence that hepatic lipase acts as a ligand for lipoprotein uptake. *J. Biol. Chem.* **273:** 1896–1903.
39. LaDu, M. J., T. M. Pederson, D. E. Frail, C. A. Reardon, G. S. Getz, and M. T. Falduto. 1995. Purification of apolipoprotein E attenuates isoform-specific binding to beta-amyloid. *J. Biol. Chem.* **270:** 9039–9042.
40. Stieger, S., S. Diem, A. Jakob, and U. Brodbeck. 1991. Enzymatic properties of phosphatidylinositol-glycan-specific phospholipase C from rat liver and phosphatidylinositol-glycan-specific phospholipase D from rat serum. *Eur. J. Biochem.* **197:** 67–73.